framework region and CDR amino acid positions;
(b) constructing a population of altered light chain variable region encoding nucleic acids comprising an acceptor variable region framework containing donor CDRs and a plurality of different amino acids at one or more framework regions and CDR amino acid positions;
(c) coexpressing said populations of heavy and light chain variable region encoding nucleic acids to produce diverse combinations of heteromeric variable region binding fragments, and (d) identifying one or more heteromeric variable region binding fragments having affinity substantially the same or greater than the donor CDR heteromeric variable region binding fragment. A method of optimizing the binding affinity of an antibody variable region is also provided. The method consists of: (a) constructing a population of antibody variable region encoding nucleic acids, said population comprising two or more CDRs containing a plurality of different amino acids at one or more CDR amino acid positions;
(b) expressing said population of variable region encoding nucleic acids, and (c) identifying one or more variable regions having binding affinity substantially the same or greater than the donor CDR variable region. The variable region populations can be heavy or light chains and can be expressed as individual populations or they can be coexpressed to produce heteromeric variable region binding fragments.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the alignment of anti-CD40 variable region and human template amino acid sequences.

Figure 2 shows binding reactivity of humanized anti-CD40 variants.

Figure 3 shows molecular modeling of anti-CD40 variant CW43.

Figure 4 shows a comparison of the quantitation of murine framework residues in active variants from two libraries.

DETAILED DESCRIPTION OF THE INVENTION

The invention is directed to a method of conferring donor CDR binding affinity onto an antibody acceptor variable region framework. The method effectively combines CDR grafting procedures and affinity reacquisition of the grafted variable region into a single step. The methods of the invention also are applicable for affinity maturation of an antibody variable region. The affinity maturation process can be substituted for, or combined with the affinity reacquisition function when being performed during a CDR grafting procedure. Alternatively, the affinity maturation procedure can be performed independently from CDR grafting procedures to optimize the binding affinity of variable region, or an antibody. An advantage of combining grafting and affinity reacquisition procedures, or affinity maturation, is the avoidance of time consuming, step-wise procedures to generate a grafted variable region, or antibody, which retains sufficient binding affinity for therapeutic utility. Therefore, therapeutic antibodies can be generated rapidly and efficiently using the methods of the invention. Such advantages beneficially increase the availability and choice of useful therapeutics for human diseases as well as decrease the cost to the developer and ultimately to the consumer.

In one embodiment, the invention is directed to methods of producing grafted heavy and light chain variable regions having similar or better binding affinity as the CDR donor variable region. When coexpressed, the grafted heavy and light chain variable regions assemble into variable region binding fragments having similar or better binding affinity as the donor antibody or variable region binding fragments thereof. The grafting is accomplished by generating a diverse library of CDR grafted variable region fragments and then screening the library for binding activity similar or better than the binding activity of the donor. A diverse library is generated by selecting acceptor framework positions that differ at the corresponding position compared to the donor framework and making a library population containing of all possible amino acid residue changes at each of those positions together with all possible amino acid residue changes at each position within the CDRs of the variable region. The grafting is accomplished by splicing a population of encoding nucleic acids for the donor CDR containing species representing all possible amino acid residues at each CDR position into a population of encoding nucleic acids for an antibody acceptor variable region framework which contains species representing all possible amino acid residue changes at the selected framework positions. The resultant population encodes the authentic donor and acceptor framework amino acid sequences as well as all possible combinations and permutations of these sequences with each of the 20 naturally occurring amino acids at the changed positions.

In another embodiment, the invention is directed to methods of producing grafted heavy and light chain variable regions, and heteromeric binding fragments